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GENE THERAPY FOR THE TREATMENT OF PITUITARY TUMORS

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Abstract

Pituitary adenomas constitute the most frequent neuroendocrine pathology in humans. Current therapies include surgery, radiotherapy and pharmacological approaches. Although useful, none of them offers a permanent cure. Current research efforts to implement gene therapy in pituitary tumors include the treatment of experimental adenomas with adenoviral vector-mediated transfer of the suicide gene for thymidine kinase, which converts the prodrug ganciclovir into a toxic metabolite. In some cases, the suicide transgene has been placed under the control of pituitary cell-type specific promoters. Also, regulatable adenoviral vector systems are being assessed in gene therapy approaches for experimental pituitary tumors. Although the efficiency and safety of current viral vectors must be optimized before clinical use, they remain as highly promising therapeutic tools.

Keywords

gene therapy; viral vectors; pituitary tumors; neurosurgery; suicide gene therapy; combined therapy; IGF-I gene therapy; proapoptotic genes

INTRODUCTION

Pituitary adenomas constitute the most frequent neuroendocrine pathology in humans, comprising up to 15% of primary intracranial tumors [1]. Also, pituitary tumors represent the most prevalent pathology in old female rats [2]. Clinical manifestations of pituitary adenomas arise from overproduction of hormones in microadenomas or from mass effects in larger tumors.

There are six types of hormone-producing pituitary adenomas which reflect the cell type that originated the tumor (Table 1). These tumor types are prolactin (PRL)- and growth hormone (GH)-secreting tumors that cause reproductive abnormalities [3] and the syndromes of acromegaly [4], respectively; corticotropin (ACTH)-producing tumors that cause Cushing's disease [4] gonadotropin- and thyrotropin-secreting tumors that cause abnormalities in their respective axes and null-cell type adenomas that produce none of the above hormones [5,6].

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Mass effects of enlarging tumors usually include visual field defects resulting from compression of the optic nerves, headaches, hypopituitarism and, rarely, invasion into the skull base causing multiple intracranial nerve palsies. Only exceptionally are pituitary tumors truly malignant with distant metastases [7].

Current therapies for pituitary tumors include surgery and radiotherapy, as well as pharmacological approaches for some types [6,8]. Transsphenoidal surgery has proven to be highly effective for microadenomas but not for macroadenomas [9]. In both cases post-surgical complications, like the appearance of a new pituitary dysfunction, diabetes insipidus and sometimes fistula formation, develop even in patients operated on by specialist surgeons [10]. Pharmacological therapies, with dopamine agonists like bromocriptine, cabergoline and quinagolide, have met with remarkable success in shrinking prolactinomas and reducing the hyperprolactinemia associated with them [11]. Unfortunately, a significant rate of side effects like nausea, vomiting, postural hypotension, dizziness, headaches, and constipation is present in long-term treatments with these drugs [12]. In patients with GH-producing adenomas, somatostatin analogues and depot preparations of octreotide can lower the usual hypersomatotropinemia to levels compatible with acceptable long-term survival [13]. Treatment with octreotide of 27 newly diagnosed acromegalic patients for 6 months, followed by treatment with a long-acting octreotide preparation for additional 6 months, resulted in 79% of the patients having mean serum GH levels below 5 mU/liter, 53% having normal IGF-I levels, and 73% of the patients showing greater than 30% tumor shrinkage [14]. None of the aforementioned pharmacological treatments cure the tumors which will usually recur if the medications are discontinued. Radiotherapy is usually used as an adjunctive treatment after pituitary tumor surgery. Although long-term irradiation is often effective to prevent tumor regrowth, it usually causes complications, the most frequent (58 to 83%) of which is hypopituitarism [15]. Other less frequent complications associated with conventional radiotherapy for pituitary adenomas are 2% optic chiasmal injury and 0.2% radiation brain necrosis. Although rare, radiation-induced fibrosarcomas and osteosarcomas have been reported [16]. Focused radiotherapy, either in the form of stereotactic multiarc radiotherapy or gamma-knife that can be applied to a small area, have the advantage to reduce damage to normal pituitary tissue and surrounding vascular and neuronal structures [7,17].

In summary, although important advances have been made in the treatment of pituitary tumors, a fully satisfactory therapy is not yet available. In this context, gene therapy appears as a potentially useful alternative for the treatment of pituitary tumors. Although research efforts to apply this methodology to the hypophysis are relatively recent, promising experimental results, discussed later in this review, have already emerged.

VIRAL VECTOR-BASED GENE DELIVERY METHODS

Although current gene delivery methods comprise viral and non-viral approaches, use of the latter for gene therapy of pituitary tumors has, to our knowledge, not been documented. Therefore, they will not be discussed. The wide use of recombinant viral vectors as gene delivery systems is due to their high efficiency for gene transfer (Table 2). In the pituitary gland, gene therapy strategies have mainly relied, up to now, on the use of adenovirus-derived vectors, although herpes-derived vectors have also been employed in some studies.

Adenoviral vectors

Adenovirus(Ad)-derived vectors have been used for both basic and therapeutic applications in the neuroendocrine system. Ads are non-enveloped DNA viruses, with a genome of 36 Kb, which possess the ability to infect a wide variety of dividing and non-dividing cells.

The cellular receptors for Ad are the high affinity receptor called CAR (Coxackie and Adenovirus Receptor), which attaches to the viral fiber knob protein [18] and the $a_vb_{3/5}$ integrins which interact with the viral penton base [19]. After endocytosis, the virus escapes from the endosomal compartment to the cytosol and docks onto the nuclear pore complex, where the viral DNA plus some associated proteins are translocated into the nucleus. There, the viral genome remains in a non-integrated (episomal) state. Adenoviral genes have been classified as early genes (which encode regulatory proteins that are expressed before DNA replication) and late genes (expressed after DNA replication and encoding structural proteins). The early E1 gene product is the main *trans*-activator of adenoviral gene transcription, including those genes needed for viral replication. This genomic region is deleted in adenoviral vectors in order to render them replication-defective and to prevent expression of the viral genome. This E1 genomic region must be complemented *in trans* (i.e., in a separate DNA) to replicate the vector, which is often done by means of a cell line, like the HEK 293, stably transfected with the deleted viral genes [20]. In some viral vectors other adenoviral genes have been deleted in order both to make more space for cloning exogenous genes and to diminish the immunogenicity and cytotoxicity of the vector. First generation recombinant adenoviral vectors usually lacked E1 and E3 genomic regions, the latter being deleted to make more space for exogenous sequences. However, despite the E1 deletion, some activation of viral gene transcription still occurs, perhaps mediated by cellular proteins with E1-like activity. Viral protein expression elicits a strong immune response, characterized by cytotoxic T lymphocytes (CTL), which eliminates the infected cells [21]. To overcome this limitation,, other genomic regions have been deleted in newer adenoviral vectors. The most promising of these are the so called 'gutless' or 'helper dependent' adenovectors, HD-Ads, (up to 35 kb of foreign DNA), in which all adenoviral genes are deleted and have only the ends of the adenoviral genome (inverted terminal repeats or ITR, which are necessary for viral DNA replication) and the encapsidation signal. As the vector cannot express any viral protein, generation of gutless vectors requires that all Ad genes be provided *in trans*, usually by coinfection with an adenovirus, called 'helper virus' [22]. Since the helper virus used to generate the gutless vector remains as a contaminant, several systems have been designed to minimize helper titres in gutless viral stocks [23]. With these multiple-deleted vectors, the risk of chance generation of replication-competent viruses is decreased, since several recombination events are required to regenerate a replication-competent Ad.

Adenoviral vectors have several advantages over other vectors (Table 2). 1) As indicated above, Ads can efficiently infect cells in replicative state as well as cells out of the cell cycle; 2) they have a capacity of up to 35 Kb of DNA (in HD-Ads), which in many instances provides enough space for transgene(s) and regulatory sequences; 3) the biology of these viruses is well known, and there is a wide range of adenoviral systems available; 4) Ads are easy to manipulate; 5) the fact that the Ad genome does not integrate into the host genome avoids the risk of insertional mutagenesis. [20].

As mentioned above, the critical limitation of adenoviral vectors comes from the usually strong host's immune response against the viral particle and the infected cells. This immune response limits the long-term expression of transgenes because CTL kill the infected cells and prevent re-administration of the vector. Current strategies to overcome Ad-induced immune responses include the use of Ad vectors carrying immuno-suppressant genes [24; also see below**]**, administration of immunosuppressant drugs like cyclosporin [25] and oral tolerization to viral antigens [26].

Herpes Simplex Virus type 1 (HSV1)-derived vectors

Another type of vectors that has been used for gene transfer in the pituitary gland corresponds to those derived from the HSV-1. This is a double stranded DNA virus with an envelope derived

from the host cell. The virus has 38 genes that are non-essential for the viral cycle and could therefore be deleted thus generating recombinant vectors with sufficient space to clone more than one transgene or regulatory sequences like specific or regulated promoters. HSV-1 can establish two types of infections: a lytic cycle or, in some neurons, a latency state. The lytic cycle is characterized by a cytopathic effect, in part due to the effect of infected cell proteins (ICP), coded for by immediate early (IE) genes. One of these ICP, ICP4, is the main factor implicated in the transition from the immediate early phase to the early phase of the lytic cycle. In the latent state, there is a very low level of viral gene transcription, which results in low levels of latency associated viral RNAs [27].

There are two types of HSV-1-derived vectors: amplicons, which will not be discussed here, and recombinant viruses. In the latter, the transgene(s) is cloned into the HSV-1 genome [for a review see 28]. There are two major types of these, called the first- and second-generation HSV-1-derived vectors. The first-generation vectors have a mutation or deletion in a single essential immediate early (IE) gene. A relevant example of these vectors is the *ts*K, which is the only type of herpes-derived vector so far used in the pituitary gñand (see below). This vector is a temperature-sensitive (*ts*) mutant of the HSV-1, which has a point mutation in the IE3 gene that hinders the replication process at 37 °C but not at 31°C. The IE3 gene was chosen because its protein product, ICP4, is the major *trans*-activator of early genes, and consequently, the expression of viral early and late genes is reduced when IE3 is inactive. The transgenes are cloned into the thymidine kinase (TK) locus under the control of an active HSV-1 IE promoter. Wild type revertants arise with a relatively high frequency during vector preparation and contaminate viral stocks with replication-competent viruses. A negative feature of first-generation herpes-derived vectors is their cytotoxicity, which stems from the fact that they still express toxic viral genes [29]. The second-generation HSV recombinant vectors have deletions in more than one gene, which makes them less cytotoxic. So far, they have not been used in the pituitary gland.

Other Viral Vector Systems

Retroviruses and lentiviruses are RNA viruses which are considered advantageous because they are integrating viruses. The ability to incorporate their DNA into the host allows for the therapeutic gene to be permanently present in the genome of the cells infected and can be passed on to future generations of that cell. Lentiviruses have an additional advantage due to their ability to transduce dividing and nondividing cells [30,31]. This unique characteristic makes them strong candidates for gene therapy. Unfortunately there are disadvantages that go along with these integrating vectors. The possibility of insertional mutagenesis is a significant concern or integration within an oncogene which could therefore lead to cancer [32,33]. With lentiviruses, the fear that recombination could lead to HIV infection in gene therapy patients is a drawback, even though the vectors have been constructed in such a way that recombining is a negligible possibility.

EXPERIMENTAL GENE THERAPY IN PITUITARY TUMORS

Reporter gene transfer studies

Initial studies with adenoviral and herpes-derived vectors demonstrated that these two vector systems could be used to efficiently transfer different types of genes into normal rat anterior pituitary (AP) cells in primary culture as well as in the corticotropic AtT_{20} and somatomammotropic GH_3 tumor cell lines [34,35]. Interestingly, neoplastic AP cells were found to be more susceptible to viral vector-mediated transduction than were normal AP cells. Include also the paper by Windeat et al, Endocrinology, it was the first report of transcriptional targeting and expression in the AP both in vitro and in vivo using the PRL promoter to drive transgene expression encoded within Ad vectors.

Direct stereotaxic injection into the sheep pituitary of Ad vectors carrying the E. *coli* β-galactosidase (∃-gal) gene under the control of either the human PRL (hPRL) or hCMV promoters led to high levels of transgene expression up to 7 days after surgery. Histologic examination of these pituitaries revealed varying degrees of inflammatory response, with periglandular fibrosis, lymphocytic infiltrate and venulitis in almost all cases [36].

Other *in vivo* studies in rats have shown that intravascular administration of adenoviral vectors, harboring the ∃-gal gene under the control of the hGH or the glycoprotein hormone ∀-subunit promoters, fail to express the transgene at pituitary level. On the contrary, stereotaxic injection of these vectors into the pituitary of rats succeeded in selectively expressing the transgene in the appropriate AP cell populations [37].

The HSV1-derived vector *ts*K was shown to be highly effective for *in vivo* transfer of the ∃-gal gene in rat pituitary prolactinomas [38,39].

Human pituitary gonadotroph and somatotroph adenoma cells were transduced *in vitro* using a human immunodeficiency virus (HIV)-type 1-derived vector encoding the enhanced green fluorescent protein (eGFP) gene under the phosphoglycerate kinase promoter [40].

Suicide gene therapy

Suicide gene therapy has been the more widely used approach to implement gene therapy for pituitary tumors, both *in vitro* and *in vivo*.

A recombinant adenoviral (RAd) vector, RAdTK, harboring the HSV-1 thymidine kinase (TK) suicide gene under the control of the human cytomegalovirus (hCMV) promoter, was used to transfer the TK gene to GH_3 and AtT_{20} cells. Incubation of RAdTK-treated GH_3 and AtT_{20} cells with the prodrug ganciclovir (which after phosphorylation by viral TK becomes toxic) caused ample destruction of the cultures [41]. In the same study, estrogen/sulpiride-induced rat prolactinomas were stereotaxically injected with the same RAdTK. Subsequent injection of the host animals with two daily i.p. doses of 25 mg ganciclovir/kg for 7 days succeeded in partially reducing AP tumor size and serum PRL levels. Gene therapy, using adenoviral vectors harboring the TK gene under the control of specific AP hormone promoters, namely the human GH and glycoprotein hormone ∀-subunit promoters, was effective for the treatment of GH_3 and ∀-subunit producing pituitary tumor cell lines *in vitro*, respectively [42]. An adenoviral vector, encoding the TK gene under the control of the hPRL promoter was also effective to induce apoptosis in GH_3 cell cultures exposed to ganciclovir. It did not significantly reduce the growth and PRL-secretory rate of estrogen-induced rat prolactinomas *in vivo* [43]. Adenoviral vectors, harboring the TK gene under both a promiscuous hCMV and the hPRL promoter, showed expression of the transgene for up to 3 months *in situ* in the normal rat anterior pituitary [44], while nude mice carrying GH_3 cell-grafted subcutaneous tumors were effectively treated with an adenoviral vector harboring the TK gene under the control of the hGH promoter [42].

A hybrid cDNA encoding a chimeric protein corresponding to HSV1-TK fused to enhanced GFP which still emits green fluorescense and retains TK suicide activity [45] was cloned into an adenoviral vector, termed RAd-$(GFP/TK)_{fus,}$ which successfully transduced experimental rat pituitary adenomas *in vivo* [46; Fig. 1]. This vector was also shown to express high fluorescence levels in cultures of the lung tumor cell line A549 and kill these cells when gancyclovir was added to the cultures (Fig. 2).

A GH cell-type specific adenoviral vector was constructed in which a stuffer DNA fragment, flanked by two loxP sequences, was placed between the hGH promoter and the diphtheria toxin gene (GHp-loxP-DT). When the GH-producing cell line GH_4 was co-transduced with this

vector and with another one harboring the expression cassette for GHp-Cre, rapid destruction of the cultures occurred. Furthermore, subcutaneous GH_4 tumors, generated in nude mice, involuted rapidly when topically injected with the above vectors in the tumor area [47].

Incubation of GH3 cells with RAd's expressing the gene for either tumor necrosis factor-α (TNFα)- or FasL, two pro-apoptotic molecules whose receptors are present on the membrane of lactotropic cells, was shown to increase apoptotic activity in these cells [48]. This study suggests that pro-apoptotic gene therapy may constitute a suitable alternative to TK gene therapy for the treatment of certain types of pituitary tumors, like prolactinamas.

The retinoblastoma mutant mouse

Another type of gene therapy strategy for the treatment of pituitary cancer is that based on the transfer of a gene(s) with the ability to rescue the normal phenotype of tumor cells. This approach has been implemented in mice heterozygous for the retinoblastoma (RB) tumor suppressor gene ($Rb^{+/-}$ mice). Such mice develop and succumb to characteristic pituitary intermediate lobe melanotroph tumors [49]. Transduction of tumor melanotrophic cells with a recombinant adenoviral vector (rAd5.R.Rb) carrying the human RB cDNA under the control of its own promoter, showed a high level of efficiency both *in vitro* and *in vivo* [50]. Furthermore, intracranial delivery of this vector to mice carrying actively growing melanotrophic tumors significantly reduced tumor growth and prolonged animal survival. Melanotrophic tumor proliferative index and apoptotic rates were markedly lowered in the rAd5.R.Rb-treated animals, which also showed growth-inhibitory dopaminergic neuron reinnervation of melanotrophic cells. [50].

Insulin-like growth factor I gene therapy

The pituitary gland produces several growth factors [51,52] that may affect the function and proliferation of pituitary cells through autocrine or paracrine action [53]. One such factor, insulin-like growth factor type I (IGF-I), which is abundant and homogeneously distributed throughout the anterior and intermediate pituitary lobes [54] has shown promise for the treatment of experimental pituitary adenomas. In effect, IGF-I gene therapy was recently reported to induce a partial regression of estrogen-induced prolactinomas in rats [46]. In this type of tumors, IGF-I gene therapy was also reported to normalize the somatotropic cell population and reduce GH secretion which is also elevated in these adenomas [55].

REGULATABLE VECTOR SYSTEMS FOR THE TREATMENT OF PITUITARY TUMORS

Two central objectives in the development of viral vectors are the achievement of cell-type specificity for transgene delivery, and the design of vectors where, once the transgene is incorporated into the target cell, its expression can be regulated by small molecules. Cell-type specificity has already been achieved in pituitary tumor cells by placing transgenes under the control of cell-type specific promoters like the GH, ∀-glycoprotein and PRL promoters for pituitary tumor cells, as noted previously.

Early inducible gene expression systems encountered limitations such as pleiotropic effects of the inducer, basal leakiness, toxicity of inducing agents and a low level of expression. Nevertheless, nontoxic, tightly-regulated control of transgene expression has been achieved with a tetracycline (Tet) gene control system [56]. Two types of Tet gene control system have been devised. A Tet-Off system is one such system. It is composed of a Tet transactivator (a hybrid protein termed tTA, which contains the Tet and DNA binding domains of the *E. coli* Tet repressor fused to VP16, an HSV-1 transactivator) which binds to *E. coli*-derived Tet-resistance operon regulatory elements embedded within a minimal CMV promoter (tetO/

minCMV). Binding of tTA to this region recruits strong cellular transcription factors thus inducing transcription of a transgene inserted downstream. When Tet is present, it binds to tTA causing the dissociation of tTA from the tetO/minCMV complex thereby inhibiting transgene expression. The second type of gene control system is the Tet-On system. Here tTA is modified by four amino acids giving rise to the rtTA transactivator which, by itself is inactive, but in the presence of the Tet analogue doxycycline (DOX), binds to the tetO/minCMV region and induces high levels of transgene expression.

A number of Tet-regulatable viral systems have now been developed, including a Tet-off dual adenoviral system for the ∃-gal reporter gene with the DNA sequence encoding the tTA transactivator placed under the control of the hPRL promoter. With this system, specific transgene expression in rodent lactotrophic cells was achieved both *in vitro* and *in vivo*. Furthermore, this expression could be turned off by administration of DOX and then back on when the antibiotic was removed [57]. A similar dual regulatable adenoviral vector system, in this case expressing tyrosine hydroxylase (TH) under the control of the hCMV promoter, has been also constructed. As TH is the rate-limiting enzyme for dopamine synthesis, the growth of both lactotrophic cell lines (GH_3 and MMQ) and estrogen-induced rat prolactinomas was significantly inhibited when co-transduced with the two vector components of the regulatable TH vector system. The expression of TH could be turned off by DOX, and back on by the removal of the antibiotic [58].

We are currently constructing a bidirectional Tet-Off RAd expressing the gene for $(GFP/TK)_{fus}$ and the gene for an analog of the antiinflammatory peptide, thymulin (Fig. 3). Adenovirally-expressed thymulin displays an exceptional longetvity of expression (for a RAd) in skeletal muscle and in the brain which is believed to be due to its antiinflammatory activity [59,60; Fig. 4]. Therefore, the above bidirectional vector could allow effective TK suicide pituitary tumor gene therapy without the inconvenience of inflammatory side effects.

POTENTIAL OF SUICIDE GENE THERAPY FOR THE TREATMENT OF HUMAN PITUITARY TUMORS

The development of gene therapy approaches for pituitary tumors is still incipient. While some of these approaches have already generated a core of results that emerge as a promising area of research for the treatment of pituitary tumors, no clinical trials have yet been documented. We are aware of only one gene therapy study using human pituitary cells. In this study, primary cultures of human lactotroph adenoma cells were successfully transduced with an adenoviral vector harboring the cDNA for human TH. The transduced cells not only expressed immunocytochemically-detectable TH, but were also shown to release significant levels of L-DOPA and dopamine which markedly reduced PRL release to the culture medium [61].

As already indicated, pituitary tumors seldom generate distant metastases. Although this feature allows the surgeon to spare normal pituitary tissue without the risk of tumor cell dissemination, neoplastic cells infiltrating the normal pituitary parenchyma may give rise to tumor regrowths. As mentioned before, pituitary tumor surgery is usually complemented with radiotherapy but, although this therapeutic combination is often effective to prevent tumor regrowth, it usually causes hypopituitarism due to excessive destruction of the normal pituitary parenchyma [62]. In this context, gene therapy appears to be a potentially superior alternative to radiotherapy for the treatment of pituitary tumors. In effect, after tumor removal, intrasurgical injection of an appropriate suicide vector into the spared pituitary parenchyma could achieve complete elimination of remaining tumor cells without affecting healthy cells. (Fig. 5). This therapeutic combination for the treatment of pituitary tumors has already been proposed [63].

EXPERT COMMENT

Although gene therapy requires further investigation of its efficiency and safety before it can be used for the treatment of pituitary diseases in humans, the versatility of this technique offers unique possibilites for the development of new therapeutic tools for a wide range of applications. These include tumor regression, supplementation of abnormal genes and normalization of hormone secretion. When pituitary tumors do not respond to current therapeutic strategies, pharmacological therapy is not well tolerated or the tumor becomes aggressive or difficult to treat over time, gene therapy could be an attractive treatment modality. Carefully designed and controlled clinical trials may assess the potential of gene therapy as a useful treatment option for currently difficult-to-treat pituitary tumors such as Cushing's or non-secreting macroadenomas.

Combining current treatments, particularly surgery, with gene therapy could potentiate the efficacy of the former and provide long-term remission and even cure for many types of pituitary tumors. Although the field will need to advance further before gene therapy may become a routine treatment for human pituitary tumors, it remains as a promising therapeutic approach for the treatment of pituitary adenomas and tumors located in the sella turcica.

FIVE YEAR PROSPECT

Possibly, the most important issue that will have to be dealt with in the next five years, before suicide gene therapy may be routinely used for the treatemnt of human pituitary tumors, is adenovector-induced inflammation. As in other organs, adenoviral vectors have been shown to cause inflammation in the pituitary gland, an adverse effect that could be prevented by using bidirectional viral vectors expressing the gene for HSV-1 TK and the gene for an antiinflamatory peptide like thymulin (see main text).

Alternatively, the use of less immunogenic viral vectors, like the adeno-associated viral vectors, could be considered for the treatment of pituitary tumors. It should also be pointed out that, although so far only the HSV1-TK-GCV suicide system has been used to implement destructive experimental gene therapy of pituitary tumors, other available toxic systems might offer advantages over the TK-GCV system. Thus, studies with recombinant Ad vectors harboring cDNAs for three toxic genes, namely those encoding the HSV1-TK, the E. *coli* cytosine deaminase and the deoxycytidine kinase, showed that cytosine deaminase had the highest efficacy to reduce the growth of different human lung carcinoma cell lines [64].

Antisense and dominant negative gene therapies may constitute alternative strategies for the treatment of pituitary tumors. Suppression of endogenous basic fibroblast growth factor (bFGF) expression by means of an Ad vector carrying an antisense transgene for bFGF caused inhibition of the proliferation of human glioma cells. The same effect was achieved by means of an Ad vector carrying a transgene for a dominant negative FGF receptor [65].

Key issues

- Pituitary adenomas constitute the most frequent neuroendocrine pathology in humans.
- Current therapies for pituitary tumors have shown a significant degree of success but in general, are neither devoid of unwanted side effects nor offer a permanent cure.
- The development of gene therapy approaches for the treatment of pituitary tumors emerges as a novel interventive startegy.

- Intrasurgical gene therapy using adenovectors expressing the suicide gene for thymidine kinase appears as promising adjunt treatment to nonradical surgical removal of pituitary tumors.
- Inflammation remains the most significant limitation of adenovirally-mediated suicide gene therapy of pituitary tumors, an adverse effect that could be prevented by using bidirectional viral vectors expressing the gene for TK and the gene for an antiinflamatory peptide, like thymulin.

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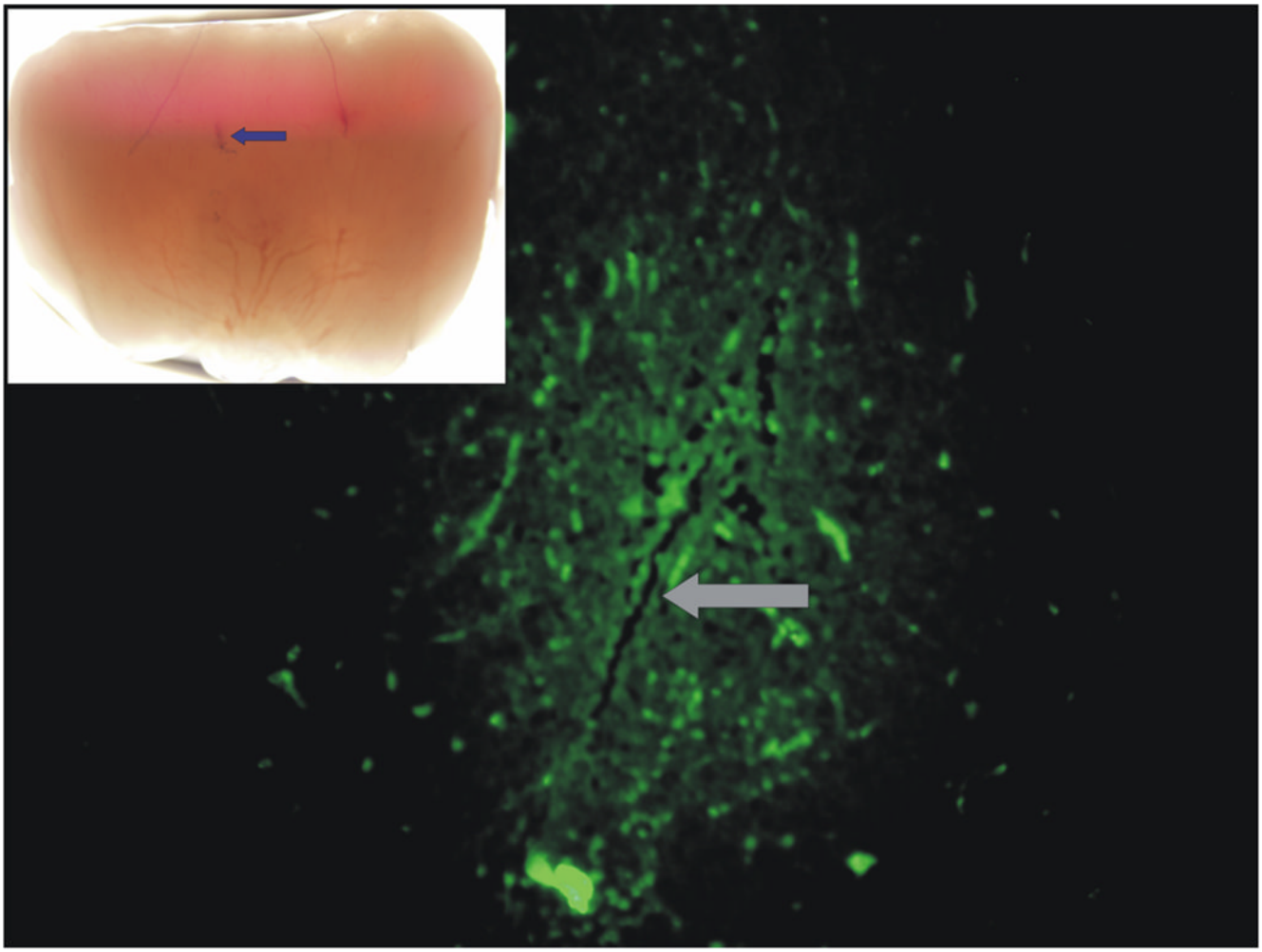

Figure 1. Expression of transgenic $(GFP/TK)_{fus}$ in a rat pituitary adenoma

The main panel shows the green fluorescence of transduced cells around the entry point (arrow) of the needle used to stereotaxically deliver RAd-$(GFP/TK)_{fus}$ into the tumor Obj. ×20. The **inset** shows a low magnification view of the same pituitary adenoma where an entry point of the needle can be seen (arrow). No structural damage is evident as a consequence of the injection. Obj. ×4 **(From Console et al., 2008, with permission)**

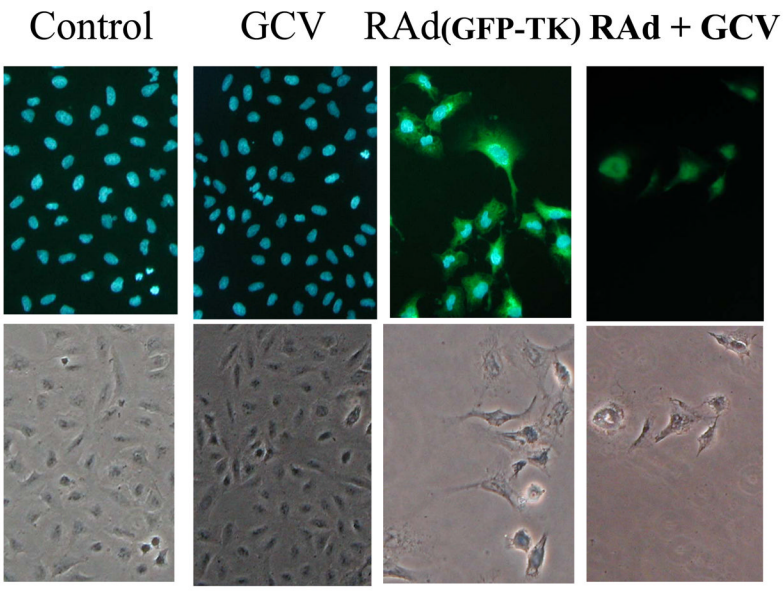

Figure 2. Suicide activity of a GFP/TK fusion protein

The suicide activity of an addenoviral vector, **RAd-(GFP/TK)$_{fus}$**, harboring a DNA sequence coding for the fusion protein **(GFP/TK)$_{fus}$**, between eGFP and HSV-1 TK was tested in the A549 human lung tumor cell line. Upper panels correspond to fluorescence images whereas lower panels correspond to phase contrast images. The leftmost panel corresponds to control cells (nuclei were labeled with DAPI and show blue fluorescence). Second panel from the left corresponds to cells incubated for 4 days with 100 μM ganciclovir (GCV), and shows that the viability of the cells was not affected by GCV alone. Third panel from the left corrsponds to cells incubated for 4 days with **RAd-(GFP/TK)$_{fus}$**. As expected green fluorescence is observed in the cytoplasm of transduced cells. The rightmost panel corresponds to cells incubated during 4 days with the vector plus 100 μM GCV. A substantial reduction in the number of cells was observed which confirms the suicide activity of the fusion protein. **(Herenu et al., unpublished)**

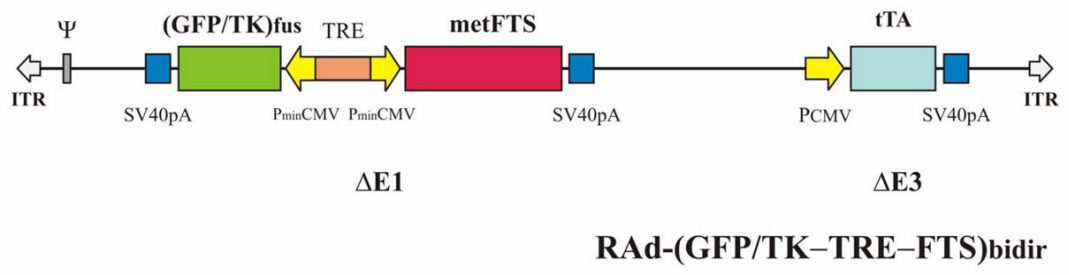

Figure 3. Proposed bidirectional Tet-Off regulatable adenovector expressing a fluorescent form of TK and the antiinflammatory peptide thymulin (FTS)

This adenovector, termed **RAd-(TK/GFP-TRE-FTS),** would combine the potent suicide activity of HSV1 TK with the antiinflammatory action of FTS, thus avoiding unwanted damage to healthy pituitary cells surrounding the tumor. Furthermore, the intrinsic green fluorescence of the suicide protein would allow ready identification of transduced pituitary cells in experimental tumors. The DOX-dependent regulatability of this system would enable the experimenter to switch transgene expression off and back on by respectively adding and removing the antibiotic DOX from the drinking water of the animals. **Figure references:** metFTS, DNA sequence coding for a thymulin analog; PminCMV, minimal cytomegalovirus promoter; TRE, tetracycline responsive element; ITR, inverted terminal repeat; Δ E1 and Δ E3, deletions in the Ad5 genome; SV40, simian virus 40 polyadenylation signal; ψ, packaging signal. **(Herenu et al., unpublished)**

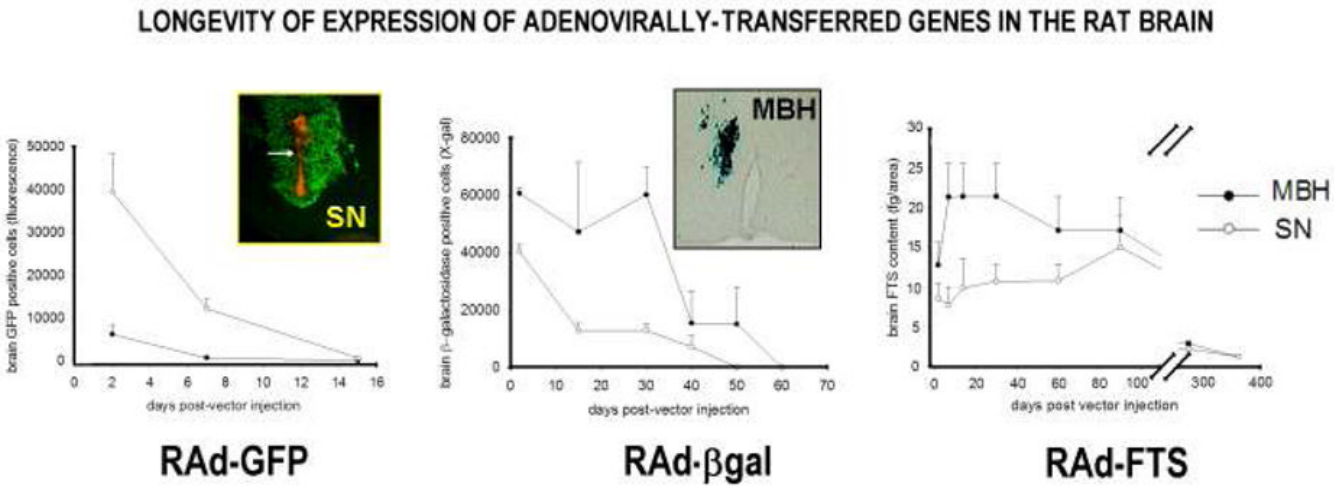

Figure 4. Differential length of expression for adenovirally delivered genes for thymulin, GFP and β-gal in the rat brain

Adenoviral vectors harboring the genes fot βgal, GFP or thymulin (FTS), were injected into de susbtantia nigra (SN) and medialbasal hypothalamus (MBH) of young rats and gene expression assessed by immunofluorescence or bioassay at different times post vector injection. Expression longevity of β-gal (left panel) and GFP (center panel) was much shorter than that of FTS (right panel) in both brain regions. Bars over symbols represent SEM. Insets show β-gal and GFP expression in the SN on post-injection day2. **(Morel et al., unpublished)**

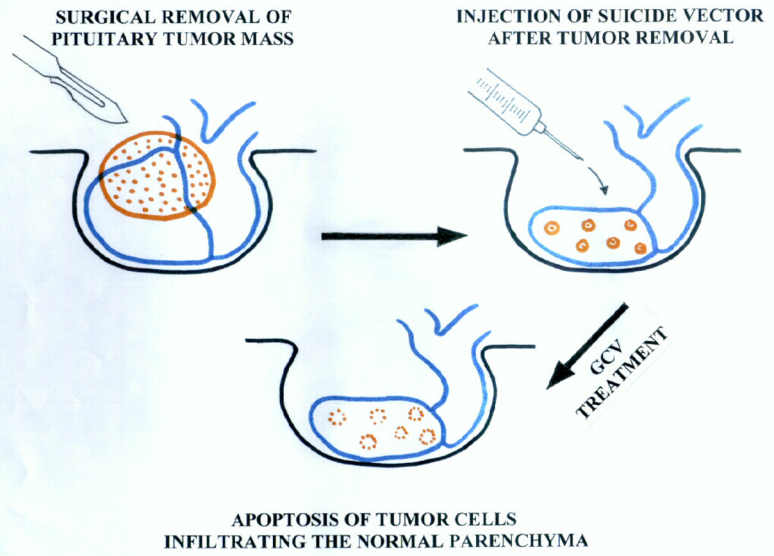

Figure 5. Schematic representation of a possible application of suicide gene therapy to complement surgical treatment of pituitary tumors

Initially the main tumor mass is removed, sparing the normal pituitary parenchyma. Subsequently, the surgeon injects in the pituitary tissue that surrounded the tumoral mass a suspension of an appropriate vector harboring the TK suicide transgene. A few days later the patient is treated with ganciclovir for a convenient period of time in order to eliminate those infiltrating tumor cells that escaped the surgical removal **(from Goya et al, 2004, with permission)**.

Table 1

Classification of Pituitary Tumors

Subtype	% of pituitary tumors	Hormone expression	Principal clinical manifestations
Prolactinomas	25–41	PRL	Galactorrhea, hypogonadism
Somatotroph adenomas	10–20	GH (GH + PRL)	Acromegalia
Corticotroph adenomas	5–15	ACTH	Cushing's syndrome (hypercortisolism)
Gonadotroph adenomas	10–15	FSH, LH, alpha subunit	menstrual irregularities, hypopituitarism, mechanical effects
Thyrotroph adenomas	0.5–2	TSH, alpha subunit	hyperthyroidism
Null-cell adenomas	5–10	None	mechanical effects, hypopituitarism

Table 2

Characteristics of different types of viral vectors.*

	Adenovirus		Herpes Simplex Virus		Retrovirus		
	1st generation RAs	**HD-Ads**	**Amplicons**	**Recombinant**	**Classical**	**Lentivirus**	**AAV**
Integration to host genome	no	no	no	no	yes	yes	yes
Maximun DNA insert size capacity	8 kb	36 kb	15 kb	Depends on how many genes have been deleted.	7 kb	7 kb	4 kb
Proliferative status of cells infected**	q and p	q and p	q and p	q and p	p	q and p	q and p
Immunogenicity in host	high	low	low	variable***	low	low	low
Long-term expression	no	yes	no	no	no	yes	yes
Preparation and manipulation	Easy	Difficult to scale up and to eliminate helper virus from stocks	Easy to manipulate. Contamination with helper virus	Easy	Easy (usually, virus producing cells are injected)	Easy	Difficult to purify and to scale up.
Advantages	Broad cell tropism, infection of dividing and quiescent cells, high titres	Broad cell tropism, infection of dividing and quiescent cells, longer transgene expression	Broad tropism, low toxicity	Broad cell tropism, large transgene capacity	Persistent transgene expression	Broad cell tropism, transduction of quiescent and dividing cells	
Hazards for clinical use	Inflammation, short-term transgene expression	Contamination with helper virus	Expression in nonneuronal cells only transient	Expression in nonneuronal cells only transient	Insertional mutagenesis	Potential infective activity	Insertional mutagenesis

* Since for each type of vector there are different variants, the characteristics indicated here correspond, in some instances, to the most used or best known variant(s).

** q= quiescent of post mitotic cells; p=proliferating cells.

*** Depends of the type of recombinant HSV vector used. First-generation vectors are highly immunogenic but latest-generation vectors are less so.